REMARKS

Applicants herein response to the office action mailed June 16, 2003 and the Notice of Appeal filed November 17, 2003. Applicants have amended claims 20-46 in order to better claim the invention. Support for the amendments can be found at 4-5 of the specification. Upon entry of this amendment, claims 20-46 will remain pending.

The claimed invention is not suggested by the prior art

On pages 2-5, the examiner rejected claims 20-27 and 40-41 as being obvious over the Sigma Chemical Catalog in view of the Thomas '591 patent, the Broze paper, the Berkner '944 patent, the Thomas '321 patent, the Turecek '968 patent and the Scopes paper. The Sigma catalog and the Thomas '591 patent were cited for disclosing the use of the inhibitor benzamidine in purifying factor VII. Broze was cited for disclosing the purification of factor VII without the presence of factor VIIa and the subsequent removal of benzamidine. The Berkner '944 patent and Turecek '968 patent were cited for methods of removing pathogens from blood products. Thomas was for disclosing the combination of factor VII with factors IX and X. Scopes was cited for disclosing the use of glycerol as a stabilizer. Applicants respectfully traverse this rejection.

On pages 6-8 of the office action, the examiner rejected claims 28-39 and 42-44 as obvious over the Turecek '620 patent in view of the Jorgensen '914 patent in view of the Goldfarb paper and the Scopes paper. The Turecek '620 patent was cited for disclosing the purification of factor VII from plasma using anion exchange columns.

The Jorgensen '914 patent was cited for disclosing the purification of factor VII from recombinant cells using anion exchange. Goldfarb was cited for disclosing protein purification using hydrogels. Scopes was cited for disclosing general protein purification protocols. Applicants respectfully traverse this rejection.

At the outset, applicants note that the examiner must show all of the recited claim elements in the combination of references that make up the rejection. When combining references to make out a *prima facie* case of obviousness, the examiner is obliged to show by citation to specific evidence in the cited references that (i) there was a suggestion/motivation to make the combination and (ii) there was a reasonable expectation that the combination would succeed. Both the suggestion/motivation and reasonable expectation must be found within the prior art, and not be gleaned from applicants' disclosure. *In re Vaeck*, 20 USPQ2d 1438, 1442 (Fed. Cir. 1991); *In re Dow Chemical Co.*, 5 USPQ2d 1529, 1531 (Fed. Cir. 1988); *W.L. Gore v. Garlock, Inc.*, 220 USPQ 303, 312-13 (Fed. Cir. 1983) (holding that is improper in combining references to hold against the inventor what is taught in the inventor's application); *see also* MPEP §§ 2142-43. Thus, the examiner must provide evidentiary support based upon the contents of the prior art to support all facets of the rejection, rather than just setting forth conclusory statements, subjective beliefs or unknown authority. *See In re Lee*, 277 F.3d 1338, 1343-44 (Fed. Cir. 2002).

When an examiner alleges a *prima facie* case of obviousness, such an allegation can be overcome by showing that (i) there are elements not contained in the references or within the general skill in the art, (ii) the combination is improper (for example, there

is a teaching away or no reasonable expectation of success) and/or (iii) objective indicia of patentability exist (for example, unexpected results). *See U.S. v. Adams*, 383 U.S. 39, 51-52 (1966); *Gillette Co. v. S.C. Johnson & Son, Inc.*, 16 USPQ2d 1923, 1927 (Fed. Cir. 1990); *Bausch & Lomb, Inc. v. Barnes-Hind/Hydrocurve*, 230 USPQ 416, 419-20 (Fed. Cir. 1986). Applicants address the rejections with these concepts in mind.

The first rejection is overcome by a review of the Sigma catalog, the Thomas '591 patent and Broze. The Sigma catalog avoids the activation of factor VII by using the powerful protease inhibitor benzamidine. Without the use of benzamidine, conventional anion exchangers used for purification would activate factor VII to form factor VIIa. Benzamidine, however, is toxic. See the enclosed Chemical Identification sheet from www.Hazard.com. The Sigma catalog does not address this problem, however.

The Thomas '591 patent and the Broze paper also use benzamidine, but state that it is removed by gel chromatography. However, removal procedures such as gel chromatography cannot completely remove benzamidine, and therefore the preparations of the Thomas '591 patent and the Broze paper will contain benzamidine. For example, the factor VII of the Thomas '591 patent is maintained in the presence of benzamidine until the activation is conducted. See column 7, lines 66-67. The claimed invention, on the other hand, minimizes the activation of factor VII **without** the use of the inhibitor benzamidine at all, while providing a "stable pharmaceutical preparation" that can remain in a ready-to-use state for an extended period of time. See specification at page 5, lines 14-18. Accordingly, the claimed invention will not have the

presence of benzamidine that would be found in the Thomas '591 patent and the Broze paper, but rather exhibits a capability for an inhibitor-free stability that is not possessed by the prior art.

It should also be noted that the authors of the Broze paper saw the need to also employ soybean trypsin inhibitor. See page 1244, first paragraph of the "RESULTS" section. Soybean trypsin inhibitor also is toxic, and is therefore unsuitable for use in humans. See page 2 of the enclosed JRH Biosciences Product Information sheet. The Broze paper does not discuss removal of the soybean trypsin inhibitor.

Applicants' invention avoids factor VII activation without the use of inhibitors like benzamidine and soybean trypsin inhibitor, and thus provides for the first time pharmacological factor VII preparations that meet today's standards for stability and safety. See applicants' specification at page 4, lines 4-5 and 15-18. The combination of references applied by the examiner do not suggest the claimed invention or provide the skilled person with a reasonable expectation that the invention could be attained. Rather, these references actually teach away from the attainment of a stable pharmaceutical preparation as claimed, and thus there can be no proper *prima facie* case. *See Ecolochem, Inc. v. Southern California Edison Co.*, 227 F.3d 1361, 1372-75 (Fed. Cir. 2000) (reasoning that prior art references cannot contain a motivation to combine to when one of the references teaches away from the combination). In view of the foregoing, applicants respectfully request withdrawal of the rejection.

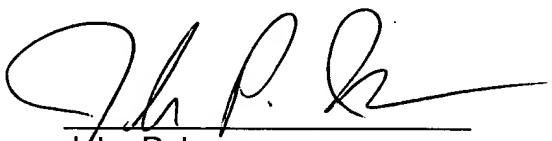
The second obviousness rejection is overcome because the Turecek '620 patent concerns the production of activated factor VII (factor VIIa) preparations, whereas the

present invention produces factor VII while minimizing the production of factor VIIa without the use of inhibitors like benzamidine and soybean trypsin inhibitor. The secondary references cannot alter this difference. Additionally, the procedures of the prior art, such as Jorgensen's anion exchangers, would only result in the autocatalytic activation of factor VII to yield activated factor VII. See applicants' specification at page 3, first paragraph. Yet the claims call for no more than 5% factor VIIa in the stable pharmaceutical preparation. Accordingly, this rejection too should be withdrawn.

Request

Applicants submit that the claims are in condition for allowance, and respectfully request favorable consideration to that effect. The examiner is invited to contact the undersigned at (202) 912-2000 should there be any questions.

Respectfully submitted,



March 17, 2003
Date

John P. Isacson
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*** CHEMICAL IDENTIFICATION ***

RTECS NUMBER : CV6126975
CHEMICAL NAME : Benzamidine, p-(aminosulfonyl)-, hydrochloride
LAST UPDATED : 198806
DATA ITEMS CITED : 3
MOLECULAR FORMULA : C7-H9-N3-O2-S.Cl-H
MOLECULAR WEIGHT : 235.71
COMPOUND DESCRIPTOR : Drug
SYNONYMS/TRADE NAMES :
* p-(Aminosulfonyl)benzamidine hydrochloride
* Benzenecarboximidamide, 4-(aminosulfonyl)-, hydrochloride
* p-Sulphonamidobenzamidine hydrochloride
* V 147

*** HEALTH HAZARD DATA ***

** ACUTE TOXICITY DATA **

TYPE OF TEST : LDLo - Lowest published lethal dose
ROUTE OF EXPOSURE : Intraperitoneal
SPECIES OBSERVED : Rodent - mouse
DOSE/DURATION : 500 mg/kg
TOXIC EFFECTS :
Details of toxic effects not reported other than lethal dose value
REFERENCE :
LANCAO Lancet. (7 Adam St., London WC2N 6AD, UK) V.1- 1823-
Volume(issue)/page/year: 2,523,1944

TYPE OF TEST : LDLo - Lowest published lethal dose
ROUTE OF EXPOSURE : Intramuscular
SPECIES OBSERVED : Rodent - mouse
DOSE/DURATION : 500 mg/kg
TOXIC EFFECTS :
Details of toxic effects not reported other than lethal dose value
REFERENCE :
LANCAO Lancet. (7 Adam St., London WC2N 6AD, UK) V.1- 1823-
Volume(issue)/page/year: 2,523,1944

TYPE OF TEST : LDLo - Lowest published lethal dose
ROUTE OF EXPOSURE : Intravenous
SPECIES OBSERVED : Mammal - cat
DOSE/DURATION : 310 mg/kg
TOXIC EFFECTS :
Vascular - BP lowering not characterized in autonomic section
REFERENCE :
JPETAB Journal of Pharmacology and Experimental Therapeutics. (Williams & Wilkins Co., 428 E. Preston St., Baltimore, MD 21202) V.1- 1909/10-
Volume(issue)/page/year: 84,160,1945

*** END OF RECORD ***

JRH BIOSCIENCES™
Accelerate Success.™

Product Information

EX-CELL™ 520
HEK 293 Serum-Free Medium

without L-glutamine

CATALOG NO. 14520

Description

EX-CELL™ 520 has been developed for the production of proteins and adenoviral vectors using the HEK 293 cell line in suspension cultures. HEK 293 cells grown in EX-CELL 520 medium grow as a single-cell suspension, as small loose aggregates that can easily be separated with gentle pipetting. Doubling times and cellular densities achieved under serum-free conditions are comparable to those achieved in a serum-supplemented culture. This medium contains Bovine Serum Albumin (BSA), as a protectant for cellular growth in spinner cultures, and low levels of recombinant growth factors.

Formulation

The formulation for EX-CELL 520 is proprietary to JRH Biosciences. Please contact Technical Services regarding specific components.

Precautions

Use aseptic technique when handling or supplementing this medium. The product is for further manufacturing use. THIS PRODUCT IS NOT INTENDED FOR HUMAN OR THERAPEUTIC USE.

Storage

Store liquid medium at 2 to 8 C, protected from light. Do not use after the expiration date.

Indications of Deterioration

Medium should be clear and free of particulates and flocculent materials. Do not use if medium is cloudy or contains precipitate. Other evidence of deterioration may include color change, pH shift or degradation of physical or performance characteristics.

Preparation Instructions

EX-CELL 520 is formulated with sodium bicarbonate and without L-glutamine. Prior to use, this medium should be supplemented with 4 mM L-glutamine by adding 20 mL/L of a 200 mM solution (JRH Catalog No. 59202). JRH recommends L-glutamine supplementation of the working volume only. Supplements, such as antibiotics, can be added to the sterilized medium using aseptic technique. Storage conditions and shelf life of the product may be affected by the nature of the supplement.

Methods for Use

Adaptation

HEK 293 cells can be adapted from cultures grown in Minimal Essential Medium (MEM) with 10% Fetal Bovine Serum (FBS) to growth in a serum-free environment using EX-CELL 520 medium. Cells grown in MEM with 10% serum should be passaged using trypsin dissociation followed by protease neutralization with a soybean trypsin inhibitor (0.1%) using the following procedure:

1. From an established HEK 293 culture (80 - 90% confluent) in MEM with 10% FBS, exchange the culture medium with EX-CELL 520 + 1% gamma irradiated FBS (JRH Catalog No. 12107).
2. Subculture using standard trypsinization techniques when the culture is 100% confluent.
3. Cells are to be kept in this low-serum concentration until normal growth rates are achieved (approximately 2 passages). HEK 293 cells cultured in EX-CELL 520 supplemented with < 1% FBS will grow in suspension and will not require any further trypsinization.

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4. After the cultures have been established in a serum-free environment, passage the cells for an additional 2 passages in EX-CELL 520 serum-free media in stationary flasks.
5. Cells can then be transferred into roller bottles (approximately 1 rpm), shaker flasks (approximately 125 - 150 rpm) or spinner flasks (approximately 75 - 125 rpm) from a starting seeding density of 2-5 x 10^5 cells/mL.

NOTE: Cells are to be handled and counted as any suspension culture. Single cells can be separated from the aggregates by gentle pipetting or by pelleting the cells using a low-speed centrifugation step followed by rapid tapping of the centrifuge tube to break up the cell pellet. Resuspend the cell suspension in an appropriate volume of fresh medium for counting and passaging.

Adaptation

Once cultures are fully adapted, the cells should be passed every 3 - 4 days at a seeding density of at least 2 x 10^5 cells/mL. An optimal seeding density should be determined by the researcher for each application and cell type.

When passing the cells, carryover should not exceed 25% of the final volume. If carryover exceeds 25%, centrifugation is recommended. Cells propagated in serum-free medium are extremely fragile. Standard techniques for centrifugation must be modified to include low-speed centrifugation to prevent damage to cells that have been propagated in serum-free medium.

During adaptation, normal trypsin concentrations may be used, but incubations should be carried out at 4 C, and exposure time should be minimal. JRH Biosciences recommends the use of a soybean trypsin inhibitor (0.1%), or sedimentation by centrifugation to remove the trypsin. Soybean trypsin inhibitor should be used with caution, as it is toxic to some cell types. Cells may also be dislodged with NO-ZYME™ (JRH Catalog No. 59226), a non-enzymatic dissociating agent.

Cryopreservation

Freezing:

Cells can be frozen in EX-CELL 520 without the reintroduction of serum.

1. Choose cultures in logarithmic growth with viabilities above 90%.
2. Prepare a freezing medium consisting of 45% cold EX-CELL 520 media, 45% spent media and 10% dimethyl sulfoxide (DMSO).
3. Centrifuge the cells at 200 *g* for 5 minutes. Remove the supernatant.
4. Resuspend the cells in the freezing medium at 5 x 10^6 to 1 x 10^7 cells/mL.
5. Rapidly transfer 1 - 2 mL of this suspension to sterile cryovials.
6. Place the vials at -20 C for 3 - 4 hours, then transfer to -70 C for 16 - 24 hours.
7. For long-term storage, transfer the vials to liquid nitrogen vapor.

Thawing:

1. Rapidly thaw a vial of frozen cells in a 37 C water bath.
2. Transfer the cells aseptically to a centrifuge tube containing 10 mL of cold EX-CELL 520 media.
3. Using low-speed centrifugation, pellet the cell suspension at 200 *g* for 5 minutes and carefully decant the supernatant without disturbing the cell pellet.
4. Resuspend the cells in 5 mL of EX-CELL 520 media.
5. Count the cells for viability and transfer to a sterile shaker flask at a seeding density of 2-4 x 10^5 cells/mL.
6. When the culture has reached a density of 1 x 10^6 cells/mL, passage the cells using standard cell culture techniques.

Characteristics

Appearance
Clear orange-red solution

Endotoxin
≤ 10.0 EU/mL

Osmolality (as supplied)
289 - 329 mOsm/kg H_2O

pH (as supplied)
7.0 - 7.4

Sterility
No microbial growth detected

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